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UREDINEOUS INFECTION EXPERIMENTS IN 1904.[2]

W. A. KELLERMAN.

The results of inoculation experiments here recorded constitute the third report of work in consecutive seasons with various species of Uredineæ. It has proved advantageous as in the previous years to attempt inoculations long in advance of the normal season for some of the species used. Not only repeated inoculations are possible the same season, thus at once removing possible doubt which is likely to arise in case of some of the successful inoculations, but this pre-season work insures non-contamination or avoidance of accidental infection, that might not be vouchsafed in case spores are in the air outside the greenhouse, the wide distribution of which in the proper season should of course always be suspected.

If testimony relative to the proposition just suggested were needed, the case of the Maize Rust related in detail below could be cited. Here the work of inoculation was begun in the middle of the winter (Jan. 16) and the successful inoculation was the starting point for an extended series of inoculations on different agricultural varieties of Maize and some other plants. When later work by another Uredinist was published which threw a shadow of doubt on some of the results, it was possible — then late in the season — to repeat the precise experiment alluded to and certainty was thereby restored. The work in detail will now be given.

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(1) Contributions from the Botanical Laboratory of the Ohio State University, XVIII.

MAIZE RUST — PUCCINIA SORGHI Schweinitz.

Inoculations with Puccinia sorghi were begun Jan. 16, 1904. Maize plants three to six inches grown in the greenhouse were used as hosts. The teleutospores were secured from infected Sweet Corn of the previous season, which had remained in the field all winter. The infections were tardy in bringing results, doubtless due in the main, to the unfavorable environment of the host plants; by this is meant that the quantity of light daily is much less than during the normal growing season in our latitude because of short days and much smoke and cloudiness in the atmosphere; and, besides, illumined as a greenhouse ordinarily is, a considerable quantity of solar light is excluded by the glass.

In the first experiment, Jan. 16, 1904, (No. 261), Sweet Corn being used as the host, a well developed uredospore sorus was discovered on the under side of one of the leaves Feb. 10, but which must have appeared several days previous.

A second inoculation with the teleutosporic material from the Sweet Corn as in the first inoculation, which was made Jan. 22 (Expt. 262) produced several pustules of uredospores which were noticed and recorded Feb. 16. Some of the sori seemed to be perhaps three days old and others were barely rupturing the epidermis; they were a bright golden color, and it was a most enchanting sight. As late as March 13 uredo-sori were found rupturing the epidermis of other host plants in the same pots and it is believed that they were also the result of the artificial inoculation with teleutospores and not from uredospores on the neighboring plants.

Careful search was made continuously for the possible appearance of spermogonia but here nor throughout the season could the slightest indication of such structures be detected. The appearance of uredospores following directly the sowing of teleutospores led to the conviction — corroborated by later experiments — that the spermogonia and æcidia are not, at least in some cases, included in the cycle of development. The inoculation having been successful in mid-winter excluded even the slightest probability that accidental infection by æcidiospores or even uredospores had obtained.

Uredospores obtained in the first experiment, where teleutospores from Sweet Corn in the field, sowed on Sweet Corn as the host, were used to inoculate Pop Corn (Expt. 280) March 30; at the same time other Sweet Corn plants were inoculated with like material. Uredospores appeared on both hosts April 11th. Here as in most cases throughout the season teleutospores on the same leaves (different sori) appeared later.

Common field corn, that is, Dent Corn, was inoculated April 20 with uredospores from the Sweet Corn. Uredo sori appeared

May 12th on the plants three or four inches high but the stalks fifteen inches high did not exhibit inoculation.

Uredospores from the Dent Corn were then used on Dent Corn both white and yellow, also on Sweet Corn and on Pop Corn (Expts. 294, 295, 296, 306, 308, 323, 324, 325, 356, 384). In all cases success attended the inoculations. It was evident by this time that we had scarcely to think of a probability of "biological" forms or varieties seeing that in case of at least three of the so-called "agricultural species" of Maize there was no invulnerability to one and the *same individual Rust-plant,* if the expression be allowed.

Effort was then made to secure all of the "agricultural species" of maize and attempt inoculations using uredospores of the several kinds of host plants now available. Several repetitions of previously outlined combinations were successfully made (Expts. 292, etc.)

On May 15th uredospores which had been harvested on yellow Dent Corn (these having been obtained by sowing the uredospores from Sweet Corn that were the result of inoculation with the teleutospores from Sweet Corn plants of the previous season, were sown on young Pod Corn plants, (Expt. 355). Sori of uredospores followed May 23. This experiment was repeated with success May 22-29 (Expt. 383).

On the same date (May 15) uredospores (of the same pedigree as those mentioned in the preceding paragraph) were sown on small plants of Flour Corn and on corresponding date, viz., May 23, uredo pustules appeared, (Expt. 357). Repetition of such inoculation was successful, date June 4-15.

For the sake of zeian chronological consistency it may be stated here that on date now mentioned, May 15, teleutospores from Sweet Corn (same as used in first experiment) were sown on Dent Corn and on May 28 uredosori appeared, (Expt. 361). This result is a confirmation of the previous conclusion (result of Expt. 261), namely, that two spore producing stages only are included in the Maize Rust life cycle — based, however, it must be remembered, on two tests only!

Again, using the uredospores obtained by the first inoculation experiments of the season, this time the host being Flint Corn, abundant success followed. In fact from this time on, many plants of Flint Corn, as well as the other kinds used as hosts, became inoculated when placed in close proximity to plants well infected by previous experiments.

It may be stated that uredospore-formation continued as long as the host-plant grew thriftily. But when the latter declined so that the leaves began to turn yellow, pustules of teleutospores appeared.

NO BIOLOGICAL SPECIES OF PUCCINIA SORGHI.

It will be noted that the six so-called "agricultural species" of Maize, namely *Zea everta* (or Pop Corn), *Zea indurata* (Flint Corn), *Zea amylacea* (Soft or Flour Corn), *Zea saccharata* (Sweet Corn), *Zea indentata* (Dent Corn), and *Zea tunicata* (Pod Corn) were used as hosts and with equally successful results. The teleutospores and uredospores were applied as detailed above. The conclusion seems to be warranted that there are no "biologic" species in case of the Maize Rust known as Puccinia sorghi Schw.

A NEW HOST FOR PUCCINIA SORGHI.

Seeds of Teosinte, Euchlæna luxurians, were furnished me by the U. S. Department of Agriculture and thirty young plants were soon available for purposes of inoculation. Using the uredospores obtained by an early inoculation of the season, making the sowings May 15, good infection was exhibited May 24, an abundance of uredospores present at that date. Another infection was made June 4 on this species as host and on June 15 a uredo pustule appeared. So far as I am aware this host has not heretofore appeared in literature.

UNSUCCESSFUL RESULTS ON OTHER HOSTS.

When Schweinitz published his description of Puccinia sorghi he said as to its occurrence: "frequens in foliis Sorghi et Zeæ." Under apparently favorable circumstances many inoculations were attempted with Sorghum vulgare, Saccharum officinarum and Tripsacum dactyloides, all of which were unsuccessful.

Roots of Tripsacum were removed to the greenhouse very early in the spring and numerous vigorous plants were available for the inoculations May 15 and on later dates (Expts. 354, 365, 380, 391, 396, 400, 405), but in no case were the efforts rewarded with success though uredospores were used from sori which furnished the inoculating material for the successful inoculation on Maize plants.

Seedlings of Sorghum vulgare, both common Sorghum and Broom Corn, were inoculated with tested uredospores and yet all the attempts failed. It appears that this host when grown in the greenhouse is invulnerable to Puccinia sorghi from Maize (Expts. 359, 360, 362, 397, 399, 403, and 404).

A living plant of Sugar Cane, Saccharum officinarum, was kindly sent me from Mississippi by Prof. S. M. Tracy. After recovery from the shock of transplanting, inoculation with uredospores similar to material used in previously successful cultures was not successful.

IS AN AECIDIUM SUPPRESSED?

An article appeared in the Botanical Gazette, 38:64-67, July 1904, entitled The Aecidium of Maize Rust, by J. C. Arthur, in which it is shown experimentally that æcidiospores from Oxalis cymosa Small placed on leaves of Maize plants produce uredospores. The following may be quoted from this author: "This prompt and very abundant appearance of the uredo could be interpreted only as the result of the æcidial infection, for corn rust had not yet appeared out of doors, and even if it had, such an unusual attack following closely within the time limit of incubation would be highly improbable. It may therefore be considered proved that the æcidium of *Puccinia Sorghi* Schw. occurs upon Oxalis, and a verification with teleutosporic material can be confidently undertaken in due time."

This result suggested that in spite of two successful inoculations with teleutospores of Maize Rust resulting in the production of uredospores (Expts. 261, 361), a mistake may have been made. Therefore a third infection experiment was undertaken July 15. Precaution was taken to grow and keep the host plants in a section of the greenhouse far removed from that portion in which the former experiments were carried on, to obviate so far as possible any accidental infection. The usual precautions were taken in making the inoculation — teleutospores from Sween Corn grown in the field the previous season being used. After nine days a pustule of uredospores put in an appearance — and thus for the third time during the period of my inoculation work extending from Jan. 16 to July 15, uredospores followed *directly* upon the application of teleutospores.

If these experiments can be relied on, we may say that while Puccinia sorghi Pers. is a heteroecious species (as proven by Dr. Arthur's experiment quoted above) it is able at times to suppress its aecidial stage. We may say besides — judging from the prevalence of the uredo and teleuto stages and the rarity of the aecidium, supposing the work of the two experimenters has really furnished results as recorded — that in the great corn growing belt of our country the aecidial stage is usually suppressed,

SUNFLOWER RUST — PUCCINIA HELIANTHI Schw.

In spite of an abundance of experimental work with Puccinia helianthi Schw. there is not a unanimity of opinion in reference to this common Rust. Whether there are two valid species, P. helianthi Schw. and P. helianthorum Schw., or only one; or whether there are several 'physiological species'; is not as yet agreed upon. Recent inoculation work leads me to think there is but one valid species and that there are no recognizable 'biologic' forms.

I was kindly furnished good teleutosporic material by Professor F. L. Stevens, collected at Raleigh, North Carolina, on

Helianthus tuberosus. Many plants of several species of Helianthus were used in the inoculations as the following account shows.

On Feb. 4, with spores from the Artichoke referred to in the preceding paragraph, the following host plant was inoculated: Helianthus annuus (Expt. 263, 264). Spermogonia appeared on March 1 and aecidia March 3. With similar teleutosporic material sowings were made April 11 on Helianthus giganteus (Expt. 282), H. tracheliifolius (Expt. 283), H. mollis (Expt. 284), H. maximiliani (Expt. 285), H decapetalus (Expt. 286), H. grosse-serratus (Expt. 287), H. orgyalis (Expt. 288), H. kellermani (Expt. 289), H. annuus (Expt. 290). Abundant spermogonia and aecidia appeared April 27 and 30 on the last named host only.

Teleutospores from Helianthus grosse-serratus furnished by Dr J. C. Arthur, Lafayette, Ind., were sown April 13 on the several hosts previously mentioned. Spermogonia and aecidia occurred only on the following hosts: Helianthus annuus (Expt. 297), H. orgyalis (Expt. 298), H. tracheiifolius (Expt. 299), H. kellermani (Expt. 300), H. giganteus (Expt. 302), H. grosse-serratus (Expt. 304), and H. decapetalus (Expt. 305). On May 17 Helianthus tuberosus (Artichoke) was inoculated — but dates and notes lost.

The above shows that Woronin's claim based on experiment that the Rust on Helianthus tuberosus was a different species (Puccinia helianthorum Schw.) from that on Helianthus annuus (Puccinia helianthi Schw.) was inaccurate. His statement as published in the Botanische Zeitung, 30:695-6, 27 Feb. 1872, is as follows: — "Endlich musste ich auch noch folgende Frage lösen: Ist die *Puccinia Helianthorum* Schw., welche Ravenel auf den Blättern der Erdbirne (*Helianthus tuberosa*) gefunden hat, eine selbständige Form, oder ist sie mit unserem Sonnenblumenrost (*Puccinia Helianthi*) identisch? Um diese Frage zo lösen, machte ich im Verlaufs zweier auf einander folgender Sommer (1869 und 1870) zahlreiche Aussaaten sowohl von *Aecidium*-Stylosporen, als auch von Uredo- und Teleutosporen der *Puccinia Helianthi* auf junge Blätter und auf ganze Pflanzen von *Helianthus tuberosus.* Das Resultat war wieder negativ; nicht ein einziges mal gelang es mir, die *Puccinia Helianthi* dem *Helianthus tuberosus* einzuimpfen. Dasselbe Resultat, wie mir aus brieflichen Mittheilungen bekannt ist, erhielt auch Prof. de Bary. Folglich muss man vermuthen, dass die Ravenel'sche Form, die *Puccinia Helianthorum,* eine ganz andere ist, als unsere *Puccinia Helianthi,* welche sich auf *Helianthus annuus* entwickelt."

I would again call attention to the uncertainty of basing too sweeping conclusions on negative results. My work with Puccinia helianthi this year has not been successful in certain cases which alone might have led me to erroneous conclusions, but the previous year these hosts responded to the inoculations! Moreover, I am ready to express the belief in view of all the work done on Puccinia helianthi by all the experimenters from Woronin down to the present, that there is but one species of Rust inhabiting the several species of Helianthus.

PINE RUST, PERIDERMIUM PINI.

Aecidiospores of Peridermium pini were obtained at Sugar Grove, Ohio, June 1st, and sowings made on Campanula americana. The plants used in the greenhouse — not being well established because of recent transference from the woods—succumbed before the normal time for appearance of any results of possible inoculation. Plants in a neighboring woods were therefore used without being removed from their natural habitat. Pustules of uredospores appeared June 8 on the leaves to which the aecidiospores were applied. The neighboring plants were free from uredo — careful search being made at the time and again at intervals later. It is therefore demonstrated that Coleosporium campanulae (Pers.) Lév. and Peridermium pini are alternate forms of one and the same species.

PUCCINIA THOMPSONII Hume.

Cultures were made with the teleutospores of this species from Carex frankii and the results were published in this Journal, p. 173 (vol. 10), but for the sake of completeness of this report the account is here reproduced:

> In default of guiding clues random cultures were made in 1903 with the teleutospores of *Puccinia thompsonii* Hume, a widely occurring rust on Carex frankii, but no success attended the attempted inoculations. Suspecting a possible connection with the Aecidium of the Elder, *Sambucus canadensis,* and noting the strong morphological resemblance between this species and the forms previously described as Puccinia bolleyana by Saccardo (1891) and Puccinia atkinsoniana by Dietel (1897),[1] attempted inoculations were renewed the present season.
>
> Partially successful results were at first discredited in spite of the strong suspicion entertained that the alternate form would prove to be none other than Schweinitz's *Aecidium sambuci.* At this time I communicated my suspicion to Dr. Arthur, also asking for good culture material in case he had any to share with me. He kindly replied

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[1] These were pronounced by Arthur to be one and the same species and the name *Puccinia sambuci* (Schw.) Arthur was applied. Cfr. Bot. Gaz. 35:15. Jan. 1903.

For convenience of reference the accepted name and synonomy may here be summarized.

Puccinia sambuci (Schw.) Arthur). Bot. Gaz. 35:15. Jan. 1903.

Aecidium sambuci Schweinitz. Trans. Am. Phil. Soc. Philadelphia, 4:294. 1834.

Puccinia bolleyana Saccardo. Am. Mon. Micr. Jour. 10:1 (fig.) Aug. 1889. Sylloge Fungorum, 9:303 (descr.) 15 Sept. 1891.

Puccinia atkinsoniana Dietel. Bull. Bull. Cornell Univ. (Science), 3:19. June 1897.

Puccinia thompsonii Hume. Bot. Gaz. 29:352. May 1900.

at once, stating that ***he had entertained such an opinion*** for nearly two years as suggested above though he had no suitable teleutospores for inoculation.

I was fortunate enough to find a small quantity of the Rust that had been exposed all winter, in the vicinity of Columbus. Proceeding with great care, most satisfactory results were obtained in a few days, when several vigorous inoculated host plants of Sambucus canadensis were rendered fairly yellow with abundant spermogonia. [Expt. 326.] In the usual time the plants exhibited the characteristic Elder aecidia — even the infection of petioles and stems causing distortions resulted from the inoculations. The evidence could not be denied by the most skeptical and I can therefore with confidence assert that Puccinia thompsonii Hume is a synonym of P. sambuci (Schw.) Arthur.

It is interesting to note, after all, that the description given by H. Harold Hume in the Botanical Gazette, 29:352, May 1900, differs in no marked or striking degree from those of P. bolleyana and P. atkinsoniana. For example, the teleutospores are said to be "oblong clavate, 46-68 x 15-24 μ," whereas in P. bolleyana they are given as clavate-oblong, 40-60 x 18-28 μ." It is said [l. c.] that this species, P. thompsonii, "somewhat resembles P. bolleyana Sacc., but differs from it in the more scattered, larger, oblong, lighter-colored sori and the somewhat longer and narrower spores." Doubtless the slight discrepancies in the three descriptions are referable to conditions or phases of a temporary character or minor importance.

UNSUCCESSFUL EXPERIMENTS.

Other Rusts were tested as Puccinia emaculata, P. osmorrhizae, P. asparagi, P. curtipes; Rusts from Carex intumescens, C. laxiflora, C. pubescens, etc., but the failures are not instructive and need not be reported in detail.

SUMMARY.

PUCCINIA SORGHI SCHW., teleutospores, was shown capable of directly producing uredospores — no spermogonia or aecidia appearing.

This Rust (uredo stage) was readily and indiscriminately transferred to the six 'agricultural species' of Maize, Zea mays amylacea, everta, indentata, indurata, saccharata, and tunicata. Hence there are no 'biologic forms' of PUCCINIA SORGHI Schw.

This species was successfully grown on Teosinte, *Euchlaena luxurians,* which is a hitherto unreported host.

Attempted inoculation of Sorghum vulgare, Saccharum offi cinarum, and Tripsacum dactyloides were unsuccessful.

RUST FROM HELIANTHUS TUBEROSUS (Artichoke) was successfully used in inoculating *Helianthus annuus,* showing that the so-called *Puccinia helianthorum* Schw. is the same as PUCCINIA HELIANTHI SCHW.

TELEUTOSPORES OF PUCCINIA THOMPSONII from Carex frankii were grown on Sambucus canadensis — showing that this Rust is PUCCINIA SAMBUCI (Schw.) Arth.

PERIDERMIUM PINI was used in a successful inoculation of Campanula americana — showing that this form is contained in the life cycle of COLEOSPORIUM CAMPANULAE (Pers.) Lév.